Regulating heart repair with cardiac-specific T lymphocytes

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Abstract
Cardiac tissue necrosis secondary to coronary artery occlusion is one of the most common and deadly sterile injuries in developed countries. In this issue of *JCI*, Rieckmann et al. identified and characterized antigen-specific $CD4^+$ T helper cells that developed in the context of myocardial infarction in mice. They showed that myosin heavy chain alpha (MYHCA) is a dominant cardiac autoantigen and that T cells with T cell receptor (TCR) specificity to MYHCA acquired a regulatory T cell (Treg) phenotype when adoptively transferred into infarcted mice, which mediated a cardioprotective healing response. Thus, Rieckmann et al. showed that an acute ischemic insult that induces sterile inflammation to the heart, promoted, rather than limited, protective T cell autoimmunity. Notably, strategies that support antigen-specific "Treg" cell response may limit the immune-inflammatory response and promote cardiac repair after acute MI.

Myocardial infarction and the inflammatory response
Cardiovascular diseases (CVD) represent a major cause of morbidity and mortality worldwide. Despite important advances in the treatment of acute MI (1), the occurrence of MI still results in left ventricular dysfunction in up to 50% of patients, which leads to the development of heart failure. Left ventricular dysfunction is the strongest predictor of adverse outcome after acute MI, and is associated with a 3 to 4-fold increase in mortality risk. In developed countries, heart failure is responsible for 1-2% of all health expenditure, which is mostly driven by repeated hospital admissions. Thus, there is a considerable need to better understand the remodelling process that follows an ischemic insult to the heart in order to limit its maladaptive components and promote a beneficial healing process.

Ischemic injury to the heart releases danger signals which alert and activate the immune system to mount a sterile inflammatory response. Both innate and adaptive immune mechanisms become involved at different stages after MI; and current knowledge suggests that suppressing excessive inflammation may limit the extension of infarct size and promote a better reparative (and potentially regenerative) response (2). But, what do we know about heart-specific adaptive T cell responses and how do the new results compare with and extend previous knowledge?

Adaptive T cell responses to myocardial injury
Self-reactive $CD4^+$ T cells are normally deleted in the thymus by a process called negative selection. Intriguingly, $CD4^+$ T cells with TCRs specific for some tissue-restricted self-antigens, including cardiac MYHCA, escape central negative selection, (1, 3) and may, therefore, make the respective tissues vulnerable to autoimmune attack. However, non-deletional tolerance

mechanisms maintain immune homeostasis. For example, under steady state conditions in heart-draining mediastinal lymph nodes (medLN), IRF8-dependent conventional dendritic cells (DCs) present cardiac MYHCA, which drive the expansion and the differentiation of MYHCA-specific $CD4^+$ T cells towards a protective Treg phenotype (4). Following acute MI, the release of pro-inflammatory cytokines (e.g., HMGB1, IL-1α, IL-1β, IL-6), along with self-antigens promote DC maturation and activation, and license MYHCA-specific T cells to adopt an (IL-17/IFN-γ) effector phenotype (4). Although this sequence may set the background for increased susceptibility to autoimmune attack post MI (5), in the absence of an autoimmune-prone background, tolerance generally remains intact and pathologic cardiac autoimmunity fails to develop (6).

Rieckmann and colleagues studied the post MI immune response in more detail. They recovered MYHCA-specific $CD4^+$ T (TCR-M) cells from MYHCA-TCR transgenic mice and showed that after MI, the transferred cells improved heart function and promoted myocardial healing (7). The authors proposed that in the post MI setting the (preferential) differentiation and expansion of MYHCA-specific $CD4^+$ T cells towards Tregs was responsible for maintaining immune tolerance and providing cardioprotective effects. This conclusion was based on an experiment in which the adoptively transferred TCR-M cells that proliferated and accumulated in the heart (but not in medLN) displayed a higher percentage of $Foxp3^+$ cells in mice with MI compared to sham-operated mice (7). In another mouse experiment, adoptively transferred T conventional (Tconv) cells converted to Treg in situ within the heart and gave rise to most of the $Foxp3^+$ TCR-M cardiac cells. However, the percentage (50%) of TCR-M Tconv cells that acquired $Foxp3^+$ expression in the ischemic heart matched sham-operated mice (7). Thus, the cardiac environment per se, whether ischemic or not, seems to favor the conversion of Tconv cells to Tregs. Given cell scarcity, no further characterization of these heart-homing TCR-M cells was pursued. Instead, after transferring cells into sham or MI mice, the authors performed gene expression profiling on medLN TCR-M cells. When cells from sham mice were compared with those from MI mice the transcription signature revealed enrichment in “pro-healing” genes. However, no specific enrichment in a Treg signature was detected; and genes associated with T helper 17 and T follicular helper cell responses were also enriched in MI medLN TCR-M cells compared with sham mice (7). Further characterization of TCR-M cells in the presence or absence of MI is warranted to determine the mechanisms responsible for their cardioprotective effects.

Rieckmann et al. also analysed the endogenous $CD4^+$ T cells that accumulate in the hearts and medLN of mice after MI. The results revealed a unique repertoire signature for cardiac T cells with preferential expansion of clones that expressed a specific TCR beta chain variable region, TCRBV-19 (7). It is interesting to note that similarly skewed T cell responses are induced after immunisation of mice with cardiac myosin or heart extract (7), and have also been described in other settings associated with cardiovascular injury, including dilated cardiomyopathy in humans (8). The reasons behind this preferential usage of TCR beta chain variable region remain unknown and merit further investigations. It will be interesting to examine whether endogenous T cell clones play beneficial or detrimental roles after MI. A recent study confirmed the occurrence of a skewed TCR repertoire in cardiac T cells of ischemic failing human hearts, indicative of tissue-specific T cell expansion (9). Cardiac T cells of ischemic failing hearts displayed memory and effector phenotypes, with a predominance of T helper type 1 and cytotoxic $CD8^+$ T cells (9), suggesting detrimental

properties. Thus, while the immediate post MI setting may still be conducive to the development of a protective Treg response (7), a failure to maintain this T cell homeostasis during chronic ischemia may contribute to the progression to heart failure. Cells equivalent to IRF8-dependent DCs ($CLEC9A^+$ DCs) and $HLA\text{-}DR^+$ macrophages showed close interactions with cardiac T cells in the failing hearts, suggesting active antigen presentation. The use of advanced single cell TCR sequencing technologies and the development of screening methods with peptide-major histocompatibility complex may help identify associated (auto)antigens.

Cardioprotective immunotherapies

Can we harness post MI $CD4^+$ T cell responses to improve patient stratification and develop cardioprotective immunotherapies? Rieckmann et al. combined computed tomography with positron-emission tomography imaging using a radiolabelled C-X-C motif chemokine receptor 4 (CXCR4) ligand ([^{68}Ga]Pentixafor) as a surrogate of T cell presence in the heart and medLN. Interestingly, medLN were significantly enlarged in patients with MI (n=22) compared to controls (n=5). Despite the small size of the latter group, and that the "controls" have been included in the study as part of an endocrinological investigation for suspected benign Conn's adenoma, the results are consistent with a local expansion of the T cell compartment in patients with MI. Additional studies are necessary to better characterize this response and examine whether it could covey any prognostic value (e.g., better healing response and improved recovery of heart function at follow-up). A recent experimental study in mice and pigs showed that controlled CXCR4 blockade through bolus injections promoted Treg mobilisation (from the spleen) and Treg homing to the heart, and enhanced their immune-regulatory properties, leading to reduced infarct size and improved heart function (10). If and when CXCR4 blockade therapy is ready for use in patients with MI, it could be interesting to evaluate CXCR4 imaging to identify the most suitable candidates.

Treg-targeted therapies have the potential to both promote cardiac repair after MI (11-13), and limit the progression of atherosclerosis (14). An interesting Treg promoting therapy currently trialled in patients with acute coronary syndromes involves administering low-dose IL-2 (15, 16), which is expected to selectively expand and activate Tregs at the expense of T effector cells (17). A big unknown, however, is whether Tregs can still be expanded and maintained locally within a hostile ischemic, necrotic and inflammatory microenvironment. The study by Rieckmann et al. reassuringly suggests that such Treg promoting therapies may effectively treat acute MI, given the apparent extraordinary ability of the cardiac environment to preserve, maintain or even promote Treg differentiation, even in the presence of sterile injury and danger signals. Nevertheless, combination with therapies that limit effector immune mechanisms (16, 18) may be necessary to ensure optimal results.

Finally, future studies should investigate why the cardiac environment is so special for promoting Treg differentiation. In this regard, it is interesting to note that fibro-adipogenic progenitors in skeletal muscle produce IL-33 to regulate muscle Treg homeostasis (19) and promote muscle repair (20). Cardiac fibroblasts are major sources of IL-33, whose production and release are further promoted by biomechanical stimuli and cell death (21). IL-33 could, therefore, at least in part, favor local differentiation, activation or maintenance of cardiac Tregs, which is a hypothesis that merits further exploration.

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References:

1. Lv H, Havari E, Pinto S, Gottumukkala RV, Cornivelli L, Raddassi K, et al. Impaired thymic tolerance to alpha-myosin directs autoimmunity to the heart in mice and humans. *J Clin Invest.* 2011;121(4):1561-73.
2. Prabhu SD, and Frangogiannis NG. The Biological Basis for Cardiac Repair After Myocardial Infarction: From Inflammation to Fibrosis. *Circ Res.* 2016;119(1):91-112.
3. Legoux FP, Lim JB, Cauley AW, Dikiy S, Ertelt J, Mariani TJ, et al. CD4+ T Cell Tolerance to Tissue-Restricted Self Antigens Is Mediated by Antigen-Specific Regulatory T Cells Rather Than Deletion. *Immunity.* 2015;43(5):896-908.
4. Van der Borght K, Scott CL, Nindl V, Bouche A, Martens L, Sichien D, et al. Myocardial Infarction Primes Autoreactive T Cells through Activation of Dendritic Cells. *Cell Rep.* 2017;18(12):3005-17.
5. Maisel A, Cesario D, Baird S, Rehman J, Haghighi P, and Carter S. Experimental autoimmune myocarditis produced by adoptive transfer of splenocytes after myocardial infarction. *Circ Res.* 1998;82(4):458-63.
6. Gottumukkala RV, Lv H, Cornivelli L, Wagers AJ, Kwong RY, Bronson R, et al. Myocardial infarction triggers chronic cardiac autoimmunity in type 1 diabetes. *Sci Transl Med.* 2012;4(138):138ra80.
7. Rieckmann M, Delgobo M, Gaal C, Buchner L, Steinau P, Reshef D, et al. Myocardial infarction favors the development of cardioprotective antigen-specific T helper cell responses. *J Clin Invest.* 2019;129(Y):xxxx-xxxx.
8. Blohm JH, Blohm N, Hummel M, Muller HH, Rohde M, Hetzer R, et al. Detection of clonal T-cell-receptor (TCR) Vbeta rearrangements in explanted dilated cardiomyopathy hearts by semi-nested PCR, GeneScan, and direct sequencing. *Med Sci Monit Basic Res.* 2013;19:111-7.
9. Tang TT, Zhu YC, Dong NG, Zhang S, Cai J, Zhang LX, et al. Pathologic T-cell response in ischaemic failing hearts elucidated by T-cell receptor sequencing and phenotypic characterization. *Eur Heart J.* 2019.
10. Wang Y, Dembowsky K, Chevalier E, Stuve P, Korf-Klingebiel M, Lochner M, et al. C-X-C Motif Chemokine Receptor 4 Blockade Promotes Tissue Repair After Myocardial Infarction by Enhancing Regulatory T Cell Mobilization and Immune-Regulatory Function. *Circulation.* 2019;139(15):1798-812.
11. Tang TT, Yuan J, Zhu ZF, Zhang WC, Xiao H, Xia N, et al. Regulatory T cells ameliorate cardiac remodeling after myocardial infarction. *Basic Res Cardiol.* 2012;107(1):232.
12. Weirather J, Hofmann UD, Beyersdorf N, Ramos GC, Vogel B, Frey A, et al. Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res.* 2014;115(1):55-67.
13. Saxena A, Dobaczewski M, Rai V, Haque Z, Chen W, Li N, et al. Regulatory T cells are recruited in the infarcted mouse myocardium and may modulate fibroblast phenotype and function. *Am J Physiol Heart Circ Physiol.* 2014;307(8):H1233-42.

14. Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, et al. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med.* 2006;12(2):178-80.
15. Zhao TX, Kostapanos M, Griffiths C, Arbon EL, Hubsch A, Kaloyirou F, et al. Low-dose interleukin-2 in patients with stable ischaemic heart disease and acute coronary syndromes (LILACS): protocol and study rationale for a randomised, double-blind, placebo-controlled, phase I/II clinical trial. *BMJ Open.* 2018;8(9):e022452.
16. Zhao TX, and Mallat Z. Targeting the Immune System in Atherosclerosis: JACC State-of-the-Art Review. *J Am Coll Cardiol.* 2019;73(13):1691-706.
17. Klatzmann D, and Abbas AK. The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. *Nat Rev Immunol.* 2015;15(5):283-94.
18. Zouggari Y, Ait-Oufella H, Bonnin P, Simon T, Sage AP, Guerin C, et al. B lymphocytes trigger monocyte mobilization and impair heart function after acute myocardial infarction. *Nat Med.* 2013;19(10):1273-80.
19. Kuswanto W, Burzyn D, Panduro M, Wang KK, Jang YC, Wagers AJ, et al. Poor Repair of Skeletal Muscle in Aging Mice Reflects a Defect in Local, Interleukin-33-Dependent Accumulation of Regulatory T Cells. *Immunity.* 2016;44(2):355-67.
20. Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, et al. A special population of regulatory T cells potentiates muscle repair. *Cell.* 2013;155(6):1282-95.
21. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, and Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest.* 2007;117(6):1538-49.